Femtosecond laser machined miniaturized micro device for serum separation in bio-sensing applications

R. INDHU[a], S. RADHA[a], E. MANIKANDAN[a], B. S. SREEJA[a], RAVI NATHURAM BATHE[b]
[a]*Department of ECE, SSN College of Engineering, Chennai– 603110, India*
[b]*International Advanced Research Centre for Powder Metallurgy and New Materials (ARCI), Hyderabad- 500005, India*

This paper presents the fabrication of miniaturized microfluidic device based on cyclic olefin polymer for the separation of serum from blood. The characteristics and performance of the proposed micro-holes array are analyzed for its trapping of red blood cell, serum separation and cholesterol presence using FEM numerical simulation. An array of micro-holes are created on biocompatible cyclic olefin polymer using femtosecond laser ablation. A single track of experiments has been carried out to obtain the required depth of 2microns with a diameter ranging from (8-27)µm for various Fluence levels of the incident laser beam. The surface morphology is characterized using optical microscopy images. The fabricated device is tested for its characteristics in real time in a clinical laboratory. The obtained results match well with the simulation results.

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1. Introduction

In human life, heart and blood vessel problems come under Atherosclerosis. Development of waxy substance in the walls of arteries leads to atherosclerosis condition. The waxy substance formed is the cholesterol which is generally found in the bloodstream, in the form of small packets (lipoprotein). The presence of cholesterol can be ascertained based on the level of lipoproteins. Cholesterol is classified into two types: Low-Density Lipoprotein(LDL) and High-Density Lipoprotein (HDL). 100-190 mg/dL is the range of low density cholesterol and 40-60 mg/dL is the range for high density cholesterol [1]. Cholesterol is detected from serum in blood by separating the clotting agents, WBC and RBC. Conventionally, in laboratories, they use the centrifuge to separate the RBC, WBC, Clotting agents to get Serum and further the level of cholesterol is identified [2]. Researchers involve different methods for the separation of bio-particles like RBC, WBC and clotting components. For the separation of such bio-particles, trending Lab on –Chip technology can be used that performs various analysis in the laboratory in miniaturized scale. This technology provides a handheld and portable single-chip device for different applications. Lab-on-Chip is capable of providing multiple analyses performed in a single chip [3]. Microfluidic is one such device developed using Lab-on-Chip technology which involves the study of manipulating and controlling fluids. The significant of using Microfluidic devices are reduced size, Handling of less amount of fluids, less consumption of chemical reagents, low power consumption, safety, reliability, portability, user-friendly. Some of the major application of Microfluidic is a Chemical synthesis, separation analysis, biodetection, and optofluidics. In Microfluidic device bio-particles can be separated based on hydrodynamic laminar flow separation, size discrimination, and non-inertia force field. The major criteria for the separation are the diameter of the bio-particle [4]. RBC, WBC and other bio-particles diameter range from 6-15µm, so the Microfluidic device has to be designed in such a way that it should separate the particles below 15µm. Ching-Chou Wou et.al, Separated RBC, WBC and other bio-particles from blood using weir-type microfilter fabricated using polydimethylsiloxane material [5].David W. Inglis, discussed the particle separation along an array of micro holes [6]. David Holmes et.al discussed the ability to sort the leucocyte particles from whole blood based on the cell size [7]. From the pieces of literature, this paper develops an Array of Micro-holes in a Microfluidic device with a dimension that is feasible to separate the bio-particles of the desired diameter.

On designing the Microfluidic chips, the material used plays a vital role in the efficiency and throughput. The materials used have gone a huge transition over these years. First glass and silicon were used in designing the Microfluidic chips and their usage provided betterment in the throughput but they did not provide long-term efficiency. The glass and the silicon materials were brittle on certain use in larger applications. They are also opaque to ultraviolet and visible lights, inaccessible fabrication, providing limited growth. PDMS overcomes both these materials since they are more flexible, optically transparent, gas and vapor permeable. PDMS also had some drawbacks as it requires some additional devices to protect them when optically transparent, gas and vapour permeable since they absorb the smaller particles on certain larger applications [8]. To overcome all the drawbacks of the above materials, Cyclic Olefin Polymer (COP) is used. Cyclic olefin polymer is a new class of polymer with high chemical resistance, low cost, high optical transparency and biocompatibility [9]. When cyclic olefin polymer is in contact with liquid, they have high

dimensional stability, low water absorption, and high mechanical stability. Based on these properties of cyclic olefin polymer it can be used for developing Microfluidic devices. Previously, these passive microfluidic devices for separation of bioparticles were fabricated using a conventional photo or soft lithography techniques. Presently, the laser machining has been attracted the field of fabricating of microscale components because of its unique characteristics such as highly directional coherence nature etc. The developments of short and ultrashort pulsed lasers dominating the field of micromachining. This technology offers simple and one step approach for fabricating three-dimensional devices [10]. In particular, the femtosecond laser is being used in creating sub-micron level features with a precise resolution due to its tight focusing, high intensity in a confined focal volume. Femtosecond laser offers unique characteristic of non-linear absorption phenomenon in transparent and dielectric materials which makes it as a useful tool for fabricating microfluidic and photonic devices. This non-linear optical absorption is independent of the material being ablated by the femtosecond laser enabling many materials like glass, silicon and polymers can be used in the fabrication of microfluidic and photonic devices [11]. Arseniy et.al have developed a large array of nanostructures for biosensing applications which utilizes femtosecond laser for nanoparticles generation and laser-induced transfer technique for the deposition process [12]. A detailed description and review of the use of femtosecond laser for fabrication of microfluidic devices for various sensing application using Glass material were reported [13]. Raffaella et.al have investigated the utilization of femtosecond laser for ablating three different polymer substrate for creating a microfluidic channel and studied the physical and chemical properties of the ablated area [14]. Recently creation of microfluidic channel in cyclic olefin polymer substrate via 1064nm nanosecond laser ablation for single and multiple passes was studied in detail. It was shown that the threshold Fluence was decreased for multiple pass laser ablation compared to single pass process [15].

In this work, we studied the formation of a large array of micro holes for separating bioparticles using femtosecond laser micromachining process. The effect of laser power on the ablation depth and crater diameter was done experimentally and discussed. It was observed that at particular fluence and scanning speed this develops a smooth and fine structured microchannel in the polymer substrate. The depth measurement and surface morphology were examined using optical profilometer and optical microscope.

2. Numerical simulation

The finite element method of simulation is carried out for the developed design using Comsol Software. The dimension of the cyclic olefin polymer is designed with 250µm x 500µm x 180µm an array of 80 Micro-holes with a diameter of 8µm. An array of Micro-holes is done in cyclic olefin polymer to separate the biological components in blood and to obtain serum. The separated serum is made to interact with cholesterol oxidase and 6-Anilinoquinoline-5,8-Quinine (Fluorescence dye).

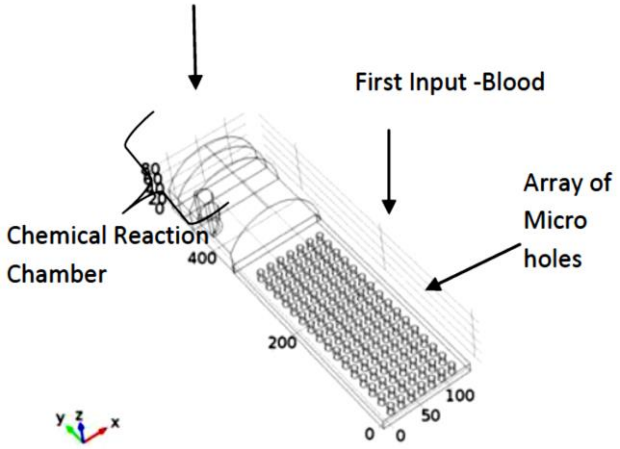


Fig. 1. Design of cyclic olefinmicrodevice in FEM simulation

The micro-holes are designed to filter all the bio-components like RBC, WBC, Clotting agents and the output obtained will be Serum. To identify the cholesterol in serum, the enzyme cholesterol oxidase is added which binds only to the cholesterol and 6-Anilinoquinoline-5,8-Quinine(Fluorescence dye) is added to identify the level of cholesterol present. In FEM Simulation, the adding of enzyme and Fluorescence dye is done in a reaction chamber which is attached to the end of the polymer film. The different level of cholesterol is identified in the reaction chamber based on the concentration obtained. The input parameters like the size of the particle (RBC, WBC, Platelets), the level of cholesterol are given manually in the simulation parameter. As the cholesterol level is varied in the parameter, there will be the change in the concentration of the fluorescence level in the reaction chamber. This explains the variation of cholesterol as it is either low density or high-density lipoprotein. The proposed micro device configuration is shown in Fig. 1. The obtained low density cholesterol and high density cholesterol contents using the proposed micro holes array using numerical simulation is shown in Figs. 2 and 3. From the Fig. 2, it is seen that the concentration in the reaction chamber is high. Since the concentration level in reaction chamber indicated the level of cholesterol, it is concluded that the obtained result gives the level of cholesterol to be high. Thus higher the value of cholesterol describes it to Low-density lipoprotein.

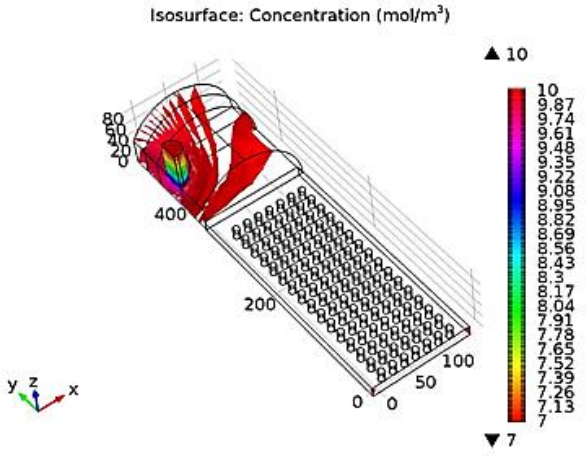


Fig. 2. Low density cholesterol

In Fig. 3, the concentration level in the reaction chamber is low and it is concluded that the obtained result gives the low level of cholesterol. Thus lower the value of cholesterol, describes it to High density lipoprotein. In simulation it has been assumed and given that, the input blood contains higher cholesterol content and the same has been observed in the outputs also. The observation from these outputs is, the intensity level of LDL (from the color bar) is comparatively higher compared to HDL level which indicates the presence of higher cholesterol level for the input blood.

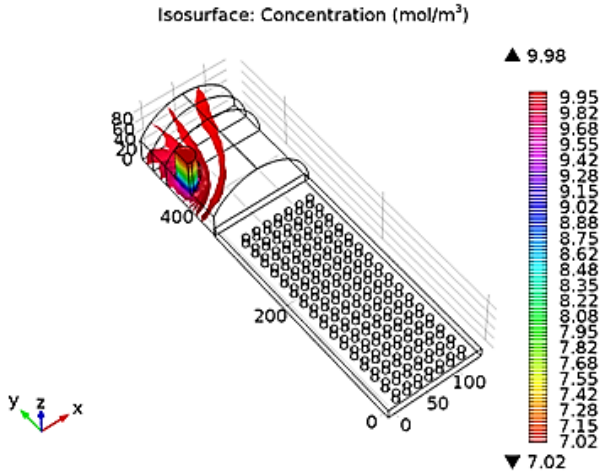


Fig. 3. High density cholesterol

3. Experiment details

3.1. Femtosecond laser machining

The specifications and the process parameters used during laser ablation process of Cyclic Olefin Polymer (COP) are shown in Tables 1 and 2. The micromachining setup used 100fs Ti: Sapphire laser as a source for ablation process. The power level of the incident laser beam was controlled by an attenuator and a half-wave plate. The incident laser beam was made to hit the target sample by using XYZ sample positioning system which provides nanometer-scale resolution. The laser Fluence is varied from 1.59J/cm^2 to 12.73J/cm^2 by adjusting the power. For the applied Fluence the corresponding crater diameter is measured by Opto-digital Microscopes (Olympus). During the entire ablation process, the scanning speed is kept constant at 90mm/sec and the measured spot size is 20µm. A single laser passes experiment has been carried to obtain the required and the minimum hole diameter. The microscopic images of the laser ablated Cyclic Olefin Polymer (COP) substrate for the applied Fluence levels are shown in Fig. 4. The crater diameter keeps on increasing as the energy of the incident laser beam increases and the threshold Fluence is calculated using equation(1), from the relationship between the applied Fluence and the crater diameter.

$$D^2 = 2d^2 \ln\left(\frac{F_0}{F_{th}}\right) \quad (1)$$

where D is the crater diameter, 2d is the beam size, F_0 applied Fluence and F_{th} is the threshold Fluence. During laser processing, the ablation process starts to occur by increasing the applied laser Fluence beyond the threshold Fluence. From equation (1) the calculated threshold Fluence for the 100fs laser pulse emitting at the 800nm wavelength is 1.49J/cm^2 which agrees well with the previously reported work. As the applied Fluence varied from 1.59J/cm^2 to 12.73J/cm^2 the crater diameter value increased from 8µm to 27µm and noticeable ablation depth is not observed in the ablation process. The plot of variation in crater diameter and ablation depth for the applied Fluence is shown in Fig. 5. It was noticed that at higher Fluence the crater diameter increases further which is not suitable for particles separation and which lead channel formation by successive ablated region. The diameter and depth obtained at a Fluence of 2.55J/cm^2 are sufficient for particles separation and this Fluence was kept constant in array fabrication.

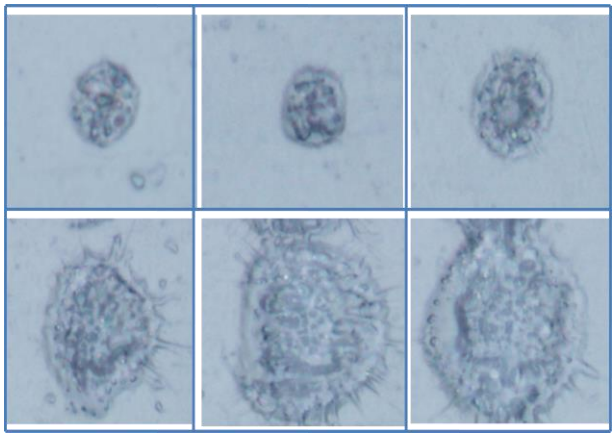


Fig. 4. Optical microscopic images of the femtosecond laser ablated holes in COP Polymer, single laser pass and various Fluence a) 1.59J/cm^2 b) 2.55J/cm^2 c) 3.18 J/cm^2 d) 6.37 J/cm^2 e) 9.55 J/cm^2 f) 12.73 J/cm^2

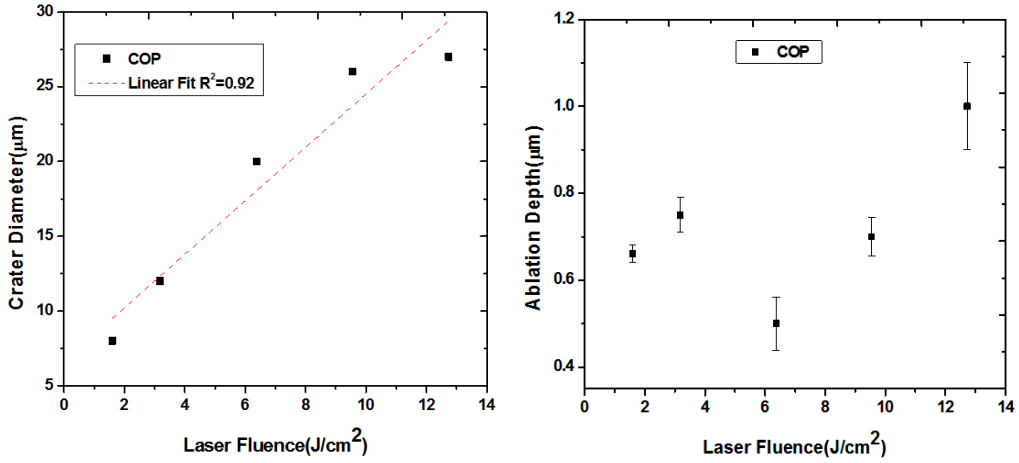
Table 1. Specifications of the femtosecond laser processing system

Wavelength	800 nm
Maximum Average Output Power	12 W
Maximum Pulse Energy	0.12J
Repetition Rate	10 kHz
Pulse width	50ps-100fs
Beam diameter	20 µm
Wavelength Supported	800, 400, 266 and 527 nm

Table 2. Process parameters used during laser processing of COP substrates

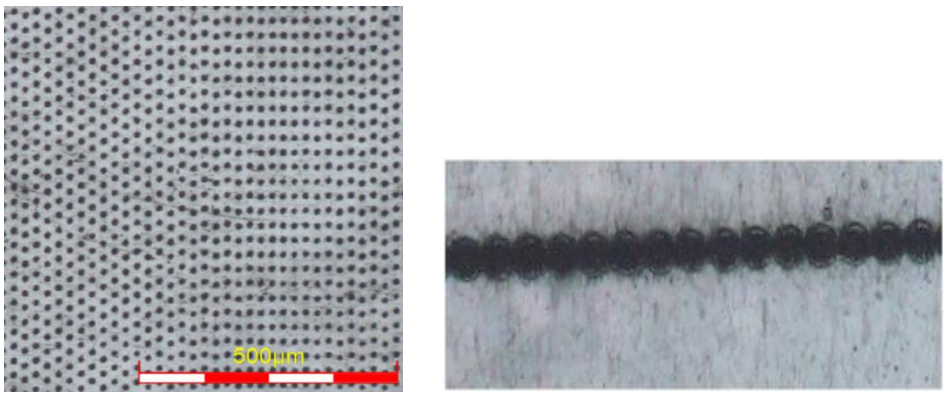
Process Parameter	Single Pass
Fluence	(1.59-12.73)J/cm^2
Scan Rate (mm/s)	90
Beam diameter (µm)	20
Pulse Width	100fs
Pulse Repetition Frequency (kHz)	10
No. of passes	1

The microscopic image of the femtosecond laser machined a large array of micro-holes is shown in Fig. 6 (a). The diameter of each hole is about 11microns which are sufficient for particles separation. These arrays of holes were fabricated at a constant Fluence of 2.55J/cm^2 and scanning speed of 90mm/sec. As discussed the microchannel was formed when ablating the polymer substrate at higher Fluence of 12.73J/cm^2 which could be utilized for microfluidic applications. The corresponding microscopic image is shown in Fig. 6 (b).



(a) *(b)*

Fig. 5. Effect of applied laser fluence on (a) crater diameter; (b) ablation depth for a single pass in COP polymer



(a) *(b)*

Fig. 6. (a) Optical microscopic image of the femtosecond laser fabricated array of micro holes for bio particles separation in COP polymer substrate; (b) Microscopic image of the developed micro fluidic channel in COP for a single pass femtosecond laser at 12.73J/cm^2 with the depth of 1µm

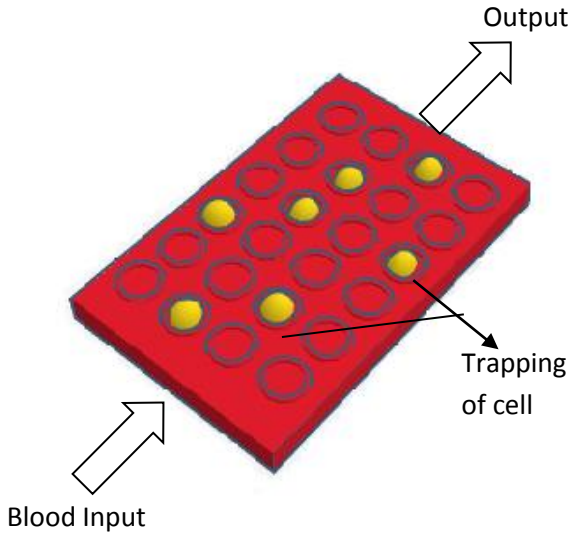


Fig. 7. Schematic representation of the real time cell trapping in the proposed micro device

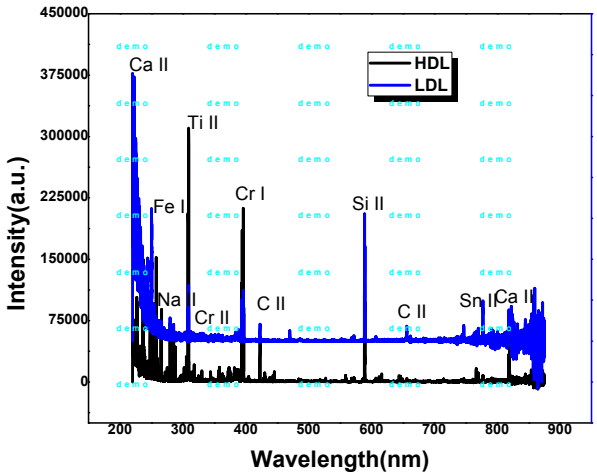


Fig. 8. Serum spectrum results using laser break down spectroscopy

In FEM numerical simulation, few microliters of blood are dropped along the array of micro-holes, and the serum gets separated on the output side. Further, it is made to react with the enzyme and fluorescents to check the level of cholesterol. A schematic view of the real time cell trapping in the proposed micro device is depicted in Fig. 7. During the real time testing the red blood cells are diluted in Dulbecco's phosphate buffered saline and made to flow through the device in order to check the number of cells gets trapped along it. 50-500 cells are diluted in one ml of buffers solution. Out of 50 cells (3-5) cells are trapped in the micro filter. Thus the fabricated device works better for cell trapping applications.

In future, if blood samples are used for testing with this device, the red blood cells and few other cells gets trapped along this device and the output obtained will be serum. The obtained serum can be given to clinical analysis to check the cholesterol level or LIBS (Laser Break Down Spectroscopy) can be applied. Many researchers are working on this technique for the detection of cholesterol level. Indhu et.al, worked on the analysis of the components present in cholesterol using laser break down spectroscopy. When the laser hit the target material, the plasma is developed and the process is captured through the collecting lens and given to spectrometer through fiber optic cable [16].With the obtained spectrum, the components present in target material can be analyzed. Based on this technique, the serum with high LDL and High HDL is taken under consideration and analyzed and it is shown in Fig. 8.

The cholesterol results obtained (HDL and LDL levels) with real-time sample agrees well to the simulated analysis. Low density and high-density concentration of cholesterol level in blood is analyzed with respect to their corresponding simulation analysis. Further, this rapidly developed and miniaturized micro device can offer a better solution for serum separation.

4. Conclusions

This work reports the design, numerical simulation, and fabrication of the miniaturized micro device of size (0.5×0.5) mm for serum separation from the blood. A single step ultrashort laser micromachining process has been utilized for fabrication process which is easier compared to conventional lithography. The effect of laser parameters such as energy, scanning speed have been studied for the cyclic olefin polymer and its effect on ablation depth and crated diameter also studied. The numerical simulation of the proposed device is done using FEM simulation method and the fabricated structure is tested for the real-time sample in the clinical laboratory. The observed results in real time match well with the numerical simulation results.

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*Corresponding author: indhur@ssn.edu.in